Kidney International, Vol. 46 (1994), pp. 97–104

Lipoprotein phospholipid composition and LCAT activity in nephrotic and analbuminemic rats

JAAP A. JOLES, NEL WILLEKES-KOOLSCHIJN, LEO M. SCHEEK, HEIN A. KOOMANS, TON J. RABELINK, and ARIE VAN TOL

Department of Nephrology and Hypertension, Utrecht University, and Department of Biochemistry, Cardiovascular Research Institute (COEUR), Erasmus University, Rotterdam, The Netherlands

Lipoprotein phospholipid composition and LCAT activity in nephrotic and analbuminemic rats. Albumin is an acceptor of lysophosphatidylcholine (LPC), product of the lecithin:cholesterol acyl transferase (LCAT) reaction, and it has been suggested that low LCAT activity and reduced cholesterol esterification rate in patients with the nephrotic syndrome may be linked to depletion of albumin. Effects of low plasma albumin levels on LCAT activity, cholesterol esterification rates and LPC-binding were therefore studied in hyperlipidemic nephrotic (NS) and analbuminemic (NAR) rats. LPC-binding was also measured in normoalbuminemic rats with dietary hypercholesterolemia. Remarkably, LCAT activity, measured with excess exogenous substrate, was not decreased but increased in both NAR and NS rats. Molar esterification rates with endogenous substrate were increased in NAR but normal in NS rats. In normoalbuminemic rats, with or without hypercholesterolemia, LPC was primarily found in the lipoprotein-deficient plasma and the HDL3 fraction. In NAR and NS rats LPC levels were increased in lipoproteins (notably in LDL and HDL2), but, in marked contrast to normoalbuminemic rats, decreased in lipoprotein-deficient plasma. Phosphatidylcholine, quantitatively the major phospholipid, was distributed proportionally over the lipoproteins in NS, NAR and control rats. Therefore, in hypoalbuminemia and analbuminemia LPC is mainly bound to lipoproteins, which is in contrast to the paucity of LPC in these particles in normoalbuminemic rats. Cholesterol esterification in nephrotic plasma is thus not impaired by lack of an acceptor for LPC-binding. The absence of an increase in molar cholesterol esterification in conjunction with increased LCAT activity points to a possible defect of the substrate for this reaction in nephrotic plasma. Increased LPC levels in LDL, a characteristic of oxidized LDL, may be a hitherto unrecognized atherosclerotic risk factor in the nephrotic syndrome.

The cholesterylesters present in plasma are derived from two sources. One part is synthesized by acyl CoA:cholesterol acyltransferase and secreted into blood from the intestine and liver in nascent lipoproteins, while another part is synthesized in plasma by lecithin:cholesterol acyltransferase (LCAT) [1, 2]. The latter enzyme transfers fatty acids from the 2-position of phosphatidylcholine (PC) to cholesterol, resulting in the formation of lysophosphatidylcholine (LPC) and cholesterol ester [1]. Cholesterol esterification in plasma contributes to the formation of larger HDL2 from small HDL3 particles [2]. The plasma cholesterol esterification process is essential for maintaining the gradient of free cholesterol between cells and HDL, which is necessary for reversed cholesterol transport: the flow of cholesterol from cells to plasma [3, 4]. Thus, cholesterol esterification may play a key role in the prevention of atherosclerosis [4].

A low HDL2 cholesterol level has been observed in the nephrotic syndrome in humans [5, 6]. The reduction in HDL2 cholesterol has been attributed to a reduction in the cholesterol esterification rate due to a reduction in LCAT activity [7, 8]. However, in one of these studies [7] LCAT activity, measured with exogenous substrate appeared normal, whereas fractional esterification of endogenous cholesterol was reduced. The latter is merely due to the high level of free cholesterol in the total lipoprotein mass in these hyperlipidemic patients, most of which is not directly available as substrate for the LCAT reaction. In a recent report LCAT activity, measured with exogenous substrate, was even found to be increased in nephrotic subjects [9]. In rats with hypoalbuminemia due to experimentally-induced nephrosis LCAT activity is also increased [10], and HDL cholesterylester levels are high [11, 12]. Thus, in the nephrotic syndrome LCAT activity measured with exogenous substrate appears to be unchanged or increased rather than decreased.

Albumin is an acceptor of lysophosphatidylcholine (LPC) product of the LCAT reaction [13]. Thus, hypoalbuminemia could theoretically disturb cholesterol esterification [7]. However, no information is available on the distribution of LPC between albumin and other potential ligands of LPC in nephrotic plasma. In plasma from non-proteinuric analbuminemic rats we observed high HDL2 levels as well as an increased cholesterol esterification rate [14]. Similarly, two patients with hereditary analbuminemia also demonstrated high LCAT activity [15]. Thus, low albumin levels as such do not appear to be linked to a defect in cholesterol esterification. If plasma cholesterol esterification is reduced in the nephrotic syndrome in the presence of increased LCAT activity measured with excess exogenous substrate [9], this must be due to a factor other than hypoalbuminemia.

In the present study we document the concentration of the phospholipids PC and LPC and their distribution in lipoproteins and lipoprotein-deficient plasma, as well as LCAT activity and cholesterol esterification rates in hypoalbuminemic plasma obtained from rats with experimental nephrotic syndrome and from analbuminemic rats. Comparing these two models enables

Received for publication May 13, 1993
and in revised form February 11, 1994
Accepted for publication February 14, 1994

separation of the effects of hypoalbuminemia *per se* from those linked to other abnormalities in plasma that are the result of proteinuria. In addition, to determine whether alterations in PC or LPC distribution in lipoproteins are related to hypoalbuminemia or are simply the consequence of hyperlipidemia, we measured phospholipid concentration and distribution in normoalbuminemic rats with hypercholesterolemia induced by dietary cholesterol supplementation.

Methods

Animals

Male Sprague-Dawley rats (N = 41) were purchased from Harlan-Olac (GB). Twenty-four were fed a standard rat chow containing 21.4% animal and vegetable protein (RMH-B^R, Hope Farms, Woerden, The Netherlands), except where otherwise specified. The remainder were fed a semisynthetic diet containing 20% casein (Hope Farms) throughout the experiment. All were allowed water *ad libitum*. The protocol was approved by the Utrecht University Board for study in experimental animals.

Nephrotic syndrome

Twelve six-week-old animals, weighing 175 to 200 g, were injected with adriamycin (Adriblastina RTU^R, Farmitalia Carlo Erba S.A., Nivelles, Belgium) into a tail vein, six at a dose of 3 mg/kg, and six at a dose of 4 mg/kg to induce nephrotic syndrome (NS). Eleven similarly aged animals were injected with an equal volume of isotonic saline. The rats treated with the low dose of adriamycin and six of the controls were fed the standard rat chow, and were sacrificed after seven months, by which time a profound proteinuria and hyperlipidemia had developed [16]. These rats were used for measuring plasma LCAT activity as well as phospholipid composition. The rats injected with the higher dose of adriamycin and the control rats were fed the semisynthetic diet and sacrificed after seven weeks. The latter group was only used for measuring plasma phospholipid composition.

Analbuminemia

Male three-month-old pathogen-free Nagase analbuminemic rats (N = 12) were derived from our own colony (which was founded with animals generously donated by Dr. S. Nagase, Tokyo, Japan). Six similarly aged Sprague-Dawley rats served as controls for the analbuminemic rats (NAR). These animals were fed the semisynthetic diet.

Dietary cholesterol

Twelve three-month-old Sprague-Dawley rats were fed either the standard rat chow (N = 4), or the standard chow supplemented with 2% dietary cholesterol (N = 4), or the standard chow supplemented with 2% dietary cholesterol and 0.5% cholic acid (N = 4). The animals were sacrificed after being exposed to these diets for 14 days.

Urine was collected in the NS rats and their controls. The rats were placed in macrolon metabolism cages, and had free access to food and water. One day was allowed for acclimatization. Subsequently two consecutive 24-hour urine samples were collected. All animals were exsanguinated in the fed state by puncture of the abdominal aorta under fentanyl-fluanisone (Hypnorm^R, Janssen, Beerse, Belgium; 0.5 ml/kg i.p.)-diazepam anesthesia. Blood was collected in chilled K-EDTA coated tubes and immediately centrifuged at 4°C for 10 minutes at 1000 × g. Plasma for cholesterol esterification analyses was stored at −80°C until analyzed. Lipoprotein phospholipid determination was performed in fresh plasma. Urinary protein was determined by the Bradford method. Plasma albumin was determined by electrophoresis. Creatinine in plasma and urine was determined colorimetrically.

Lecithin: cholesterol acyltransferase (LCAT) activity measured with endogenous or excess exogenous substrates

Plasma LCAT activity was determined by two different procedures, one of which utilizes endogenous substrate and the other excess exogenous substrate. The molar rate of cholesterol esterification refers to the esterification rate of endogenous unesterified cholesterol in plasma. For this assay undiluted plasma was incubated for one hour at 37°C. The esterification rate is constant during this period and is expressed as nmol cholesterol esterified/ml plasma/hr [17].

The plasma level of active LCAT was determined using excess exogenous substrate, containing [^{3}H]cholesterol, as described [18]. Incubations were for six hours at 37°C in a total volume of 0.145 ml, using 0.01 to 0.02 ml of rat plasma. The reaction was stopped by addition of 0.30 ml of methanol. Subsequently the lipids were extracted twice with 0.4 ml hexane. Unesterified and esterified cholesterol were separated using disposable silica columns, and [^{3}H] cholesterylesters were eluted with 3.0 ml hexane:diethylether (6:1, vol/vol) [19] and counted in a liquid scintillation counter. The measured activities are linear with the amount of rat plasma used and the within-day coefficient of variation of the LCAT assay using exogenous substrate is 4.5%. The assays were performed in duplicate and the measured activities are related to the activities in a human reference plasma pool that was included in each run. It should be noted that the measured LCAT activity using exogenous substrate reflects the activity of the enzyme as such, and is independent of the endogenous lipoproteins present in the plasma sample.

Lipoprotein isolation by density-gradient ultracentrifugation

Plasma lipoproteins were separated by density-gradient ultracentrifugation into seven fractions (chylomicrons and VLDL, d < 1.006 g/ml; intermediate density lipoprotein, IDL, d 1.006 to 1.019 g/ml; low density lipoprotein, LDL1, d 1.019 to 1.04 g/ml; LDL2, 1.04 to 1.063; high density lipoprotein, HDL2, d 1.063 to 1.125 g/ml; HDL3, d 1.125 to 1.21 g/ml and lipoprotein-deficient plasma (LDP) with a d > 1.21 g/ml) [14]. Three experimental and three control samples were analyzed in each run. Cholesterol and phospholipid concentrations were assayed enzymatically using kits (respectively Boehringer GmbH, Mannheim, Germany and bioMérieux, Marcy-l'Etoile, France). Recovery of cholesterol and phospholipid after ultracentrifugation was 104.9 ± 1.0 and 105.8 ± 3.3%, respectively.

Phospholipid composition of lipoproteins and lipoprotein-deficient plasma analyzed by thin-layer chromatography

Lipoproteins derived from plasma from two to three animals in each group were pooled depending on the phospholipid

concentration. Phospholipids were extracted in a stepwise fashion. First with a mixture of methanol (2.53 × fraction volume) and chloroform (1.27 × fraction volume), then with chloroform and water (both 1.27 × fraction volume). The chloroform fraction was then evaporated, and the residue dissolved in 2 ml chloroform:methanol (2:1, vol:vol). Phosphorus content was determined with a modified Bartlett procedure [20]. Phospholipid species were separated by thin-layer chromatography. An aliquot containing 300 nmol phosphorus was evaporated, and the residue dissolved in 100 μl chloroform: methanol (2:1, vol:vol) and spotted onto the plate. Separation of phospholipid species was achieved using a solvent composed of chloroform:methanol:acetic acid:water (100:50:16:4, vol:vol). Phospholipids were visualized with iodine, scraped from the plate, and phosphorus content of the various species (lysophosphatidylcholine, LPC, sphingomyelin, phosphatidylcholine, PC, and phosphatidylethanolamine) in each lipoprotein was determined with the modified Bartlett procedure [20].

Calculations and statistical analysis

The data derived from all rats (NS, NAR and controls) fed the semisynthetic diet were compared. Data from the NS rats injected with the low and high doses of adriamycin were averaged as the degree of proteinuria at the time of sacrifice was similar (*vide infra*). A separate analysis was performed on the data derived from the animals fed the standard rat chow, with or without cholesterol supplementation. Phosphorus concentrations of each phospholipid were summed for all lipoproteins and LDP, and the percentage of total phosphorus content of this phospholipid in each particular fraction was calculated. The LCAT activity and molar esterification rate were expressed as a percentage of the mean value found in the matched controls that were analyzed in the same run. Comparisons were performed using analysis of variance and a *t*-test with Bonferroni correction for multiple comparisons where necessary. All results are expressed as mean ± SEM.

Results

Lecithin:cholesterol acyltransferase (LCAT) activity measured with endogenous or excess exogenous substrates

Nephrotic syndrome. Urinary protein excretion in NS rats injected with the low dose of adriamycin was 563 ± 76 mg/day as compared to 76 ± 16 mg/day in the controls ($P < 0.001$). Creatinine clearance was reduced by about 20% in the NS rats. LCAT activity levels using excess exogenous substrate were increased by about 70% in the NS rats versus controls ($P < 0.01$), but plasma esterification rates were not different between these two groups (Table 1). The high levels of unesterified cholesterol in the NS rats (2.08 ± 0.25 mmol/liter vs. 0.77 ± 0.04 mmol/liter in the controls; $P < 0.01$) gave rise to reduced fractional esterification rates, despite the absence of differences in the absolute rate.

Analbuminemia. In the NAR rats both LCAT activity measured with excess exogenous or with endogenous substrates (plasma esterification rate) were significantly increased versus controls ($P < 0.01$) and versus the values found in the NS rats ($P < 0.01$; Table 1). The high levels of unesterified cholesterol in the NAR (6.62 ± 0.21 mmol/liter vs. 1.83 ± 0.04 mmol/liter in the controls; $P < 0.01$) gave rise to reduced fractional esterification rates, despite the increase in the absolute rate. The difference in unesterified cholesterol (see above) between the two groups of control rats is due to differences in age and diet.

Table 1. LCAT activity (exogenous substrate) and endogenous cholesterol esterification rates (CER) in rats with chronic adriamycin nephrosis (NS), analbuminemic rats (NAR) and control rats (CON)

	N	LCAT (% of CON)	CER (% of CON)
CON	6	100 ± 3	100 ± 6
NS	6	174 ± 16[a]	91 ± 11
CON	6	100 ± 4	100 ± 6
NAR	6	232 ± 13[ab]	174 ± 19[ab]

Samples from the experimental animals were analyzed together with those obtained from controls matched for age and diet. Data are mean ± SEM.
[a] $P < 0.01$ vs. CON
[b] $P < 0.01$ vs. NS

Table 2. Albumin, cholesterol and phospholipid concentration (mmol/liter) and phospholipid composition in plasma (% of total) in rats with chronic adriamycin nephrosis (NS), analbuminemic rats (NAR) and control rats (CON)

	CON	NS	NAR
N	11	12	12
Albumin *g/liter*	45.7 ± 1.3	19.0 ± 3.1[b]	<0.1[bd]
Cholesterol *mmol/liter*	2.5 ± 0.2	10.5 ± 1.6[b]	5.5 ± 0.4[ad]
Phospholipid *mmol/liter*	1.9 ± 0.1	5.7 ± 0.7[b]	3.5 ± 0.2[c]
N (pooled samples)	4	4	4
Lysophosphatidylcholine %	35.2 ± 2.3	27.5 ± 1.0[a]	17.1 ± 0.4[bd]
Sphingomyelin %	9.3 ± 1.9	16.2 ± 2.2	8.9 ± 0.6[c]
Phosphatidylcholine %	54.9 ± 0.8	53.1 ± 3.1	70.6 ± 0.9[bd]
Phosphoethanolamine %	0.8 ± 0.1	3.2 ± 0.4	3.5 ± 0.3[a]

Data are mean ± SEM.
[a] $P < 0.05$ vs. CON
[b] $P < 0.01$ vs. CON
[c] $P < 0.05$ vs. NS
[d] $P < 0.01$ vs. NS

Phospholipid composition of lipoproteins and lipoprotein-deficient plasma

Nephrotic syndrome. Urinary protein excretion in NS rats injected with the high dose of adriamycin was 765 ± 47 mg/day as compared to 44 ± 5 mg/day in the controls ($P < 0.001$). Creatinine clearance was reduced by about 50% in these NS rats. Plasma albumin levels were considerably reduced in all the NS rats (Table 2). Total plasma cholesterol and phospholipid concentrations in NS rats as compared to controls were increased ($P < 0.01$; Table 2). Cholesterol levels were higher than controls in all lipoproteins ($P < 0.05$ except for IDL; Fig. 1). Phospholipid levels showed a pattern similar to that of cholesterol, being higher than controls in all lipoproteins ($P < 0.05$), except in HDL3 and the LDP fraction (Fig. 2). The relative amount of LPC in whole plasma was slightly lower in NS than in controls ($P < 0.05$). The relative amounts of PC, sphingomyelin and phosphoethanolamine were not significantly altered in nephrotic as compared to control plasma (Table 2).

PC, the major phospholipid species (Table 2), peaked in LDL2 and HDL2 in both NS and control rats (Fig. 3). A similar

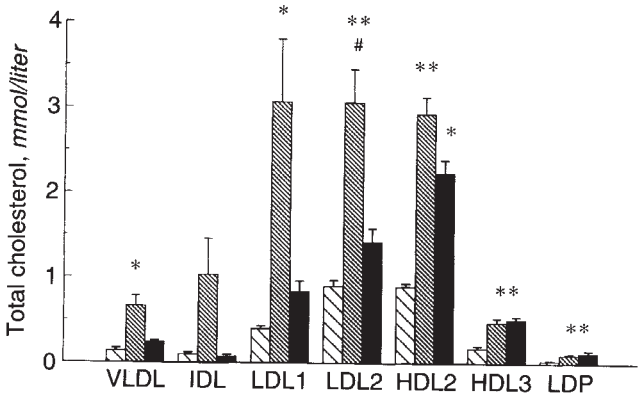

Fig. 1. *Total cholesterol levels (mmol/liter) in lipoproteins and lipid deficient plasma of control (CON, ▨, n = 12), nephrotic (NS, ▨ N = 12) and analbuminemic (NAR, ■, N = 5) rats.* Data are mean ± SEM. Abbreviations are: VLDL, very low density lipoprotein; IDL, intermediate density lipoprotein; LDL, low density lipoprotein; HDL, high density lipoprotein; LDP, lipoprotein-deficient plasma. *$P < 0.05$ vs. CON; **$P < 0.01$ vs. CON; #$P < 0.05$ vs. NAR; ##$P < 0.01$ vs. NAR.

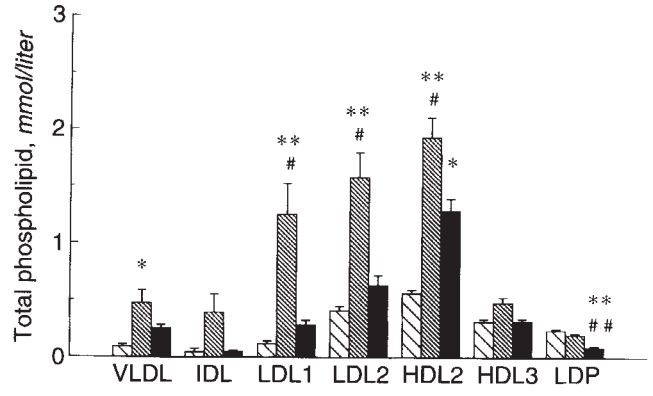

Fig. 2. *Total phospholipid levels (mmol/liter) in lipoproteins and lipid deficient plasma of control (▨, N = 12), nephrotic (▨, N = 12) and analbuminemic (■, N = 5) rats.* Data are mean ± SEM. Abbreviations and symbols are in Fig. 1.

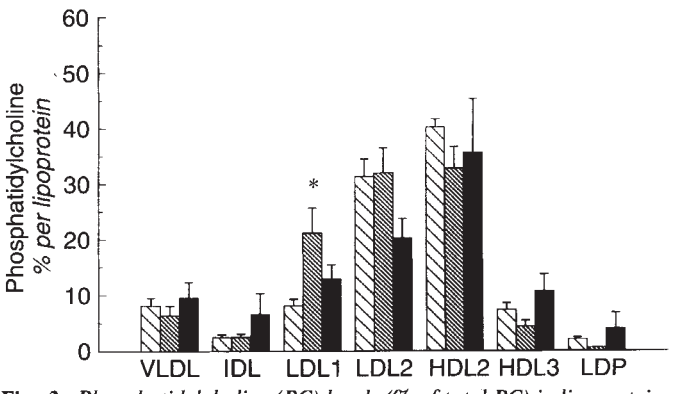

Fig. 3. *Phosphatidylcholine (PC) levels (% of total PC) in lipoproteins and lipid deficient plasma of control (▨), nephrotic (▨) and analbuminemic (■) rats.* Data are mean ± SEM of 4 pooled samples in each group. Abbreviations and symbols are in Fig. 1.

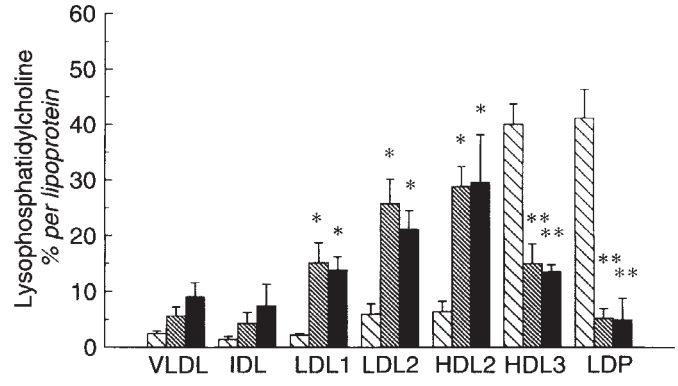

Fig. 4. *Lysophophatidylcholine (LPC) levels (% of total LPC) in lipoproteins and lipid deficient plasma of control (▨), nephrotic (▨) and analbuminemic (■) rats.* Data are mean ± SEM of 4 pooled samples in each group. Abbreviations and symbols are in Fig. 1.

pattern was observed for the minor phospholipids, sphingomyelin and phosphatidylethanolamine (not shown). The only significant difference between NS and control rats was an increase in the LDL1 fraction in the NS rats ($P < 0.05$). LPC peaked in HDL3 and the LDP fraction in control rats. In these fractions LPC levels were strongly decreased in the NS rats (both $P < 0.01$). However, in the LDL1, LDL2 and HDL2 fractions LPC showed a marked increase in the NS rats (all $P < 0.05$; Fig. 4). Consequently, LPC was five times as high in LDL1 and LDL2 in the NS rats as compared to LDL1 and LDL2 in the control rats. Note that part of the LPC content of the HDL3 fraction in the CON rats is probably due to contamination by albumin from the adjacent LDP fraction [14].

Analbuminemia. Albumin could not be detected in plasma of NAR rats. Total cholesterol and phospholipid concentrations were increased as compared to controls, but lower than in NS (both $P < 0.05$; Table 2). Lipoprotein cholesterol levels were intermediate between NS and control rats, the highest level being found in the HDL2 fraction ($P < 0.05$ vs. control; Fig. 1). Phospholipid levels were increased slightly in the VLDL and both LDL fractions, and significantly ($P < 0.05$ vs. control) in the HDL2 fraction. However, in the LDP fraction there was a marked reduction of phospholipid content in the NAR ($P < 0.01$ vs. both control and NS; Fig. 2). The relative amounts of LPC in whole plasma were decreased in NAR as compared to both control and NS rats ($P < 0.01$), whereas that of PC was increased as compared to control and NS rats ($P < 0.01$; Table 2). The relative amount of phosphoethanolamine was higher in NAR as compared to control plasma ($P < 0.05$; Table 2).

PC peaked in LDL2 and HDL2 in NAR rats as was also observed in NS and control rats (Fig. 3). In NAR LPC was significantly increased in both LDL and the HDL2 fraction (all $P < 0.05$) and markedly decreased in the HDL3 and LDP fractions (both $P < 0.01$; Fig. 4). Consequently, LPC was four to five times as high in LDL in the NAR as compared to LDL in the control rats. There were no significant differences between NAR and NS rats in the relative distribution of either PC or LPC.

Dietary cholesterol. Dietary supplementation with cholesterol with or without cholic acid did not affect plasma albumin levels (Table 3). Dietary supplementation with 2% cholesterol had no effect on total plasma cholesterol and phospholipid levels, whereas the addition of 0.5% cholic acid caused a

Table 3. Albumin, cholesterol and phospholipid concentration (mmol/liter) and phospholipid composition in plasma (% of total) in rats fed standard rat chow (control), or rat chow supplemented with 2% cholesterol, or rat chow supplemented with 2% cholesterol and 0.5% cholic acid

	Diet		
	Control	2% Chol.	2% Chol. + 0.5% chol. ac.
N	10	4	4
Albumin *g/liter*	39.3 ± 0.6	38.8 ± 0.2	39.2 ± 0.6
Cholesterol *mmol/liter*	2.35 ± 0.10	2.87 ± 0.24	8.81 ± 1.13[bc]
Phospholipid *mmol/liter*	1.63 ± 0.09	1.47 ± 0.06	2.40 ± 0.18[bc]
N (pooled samples)	3	2	2
Lysophosphatidylcholine %	34.7 ± 1.7	25.9 ± 2.8	27.4 ± 4.1
Sphingomyelin %	10.4 ± 0.7	6.3 ± 0.5[a]	6.9 ± 1.8
Phosphatidylcholine %	53.5 ± 2.0	63.1 ± 2.0	63.7 ± 3.1
Phosphoethanolamine %	1.5 ± 0.4	4.7 ± 1.4	2.1 ± 0.8

Data are mean ± SEM.
[a] $P < 0.05$ vs. control
[b] $P < 0.01$ vs. control
[c] $P < 0.01$ vs. cholesterol only

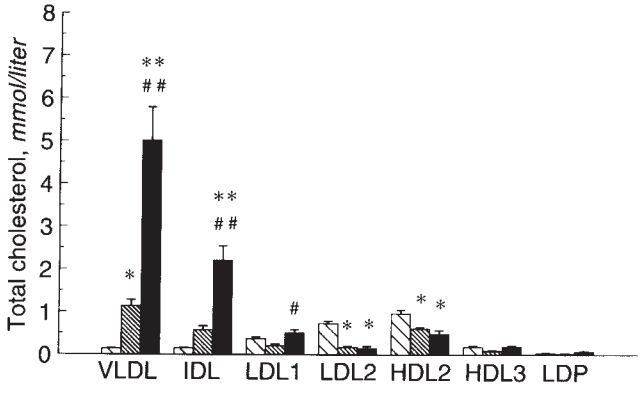

Fig. 5. *Total cholesterol levels (mmol/liter) in lipoproteins and lipid deficient plasma of rats fed standard rat chow (▨, N = 6), rats fed rat chow supplemented with 2% dietary cholesterol (▩, N = 4), and rats fed rat chow supplemented with 2% dietary cholesterol plus 0.5% cholic acid (■, N = 4).* Data are mean ± SEM. Abbreviations are in Fig. 1. *$P < 0.05$ vs. CON; **$P < 0.01$ vs. CON; #$P < 0.05$ vs. 2% dietary cholesterol; ##$P < 0.01$ vs. 2% dietary cholesterol.

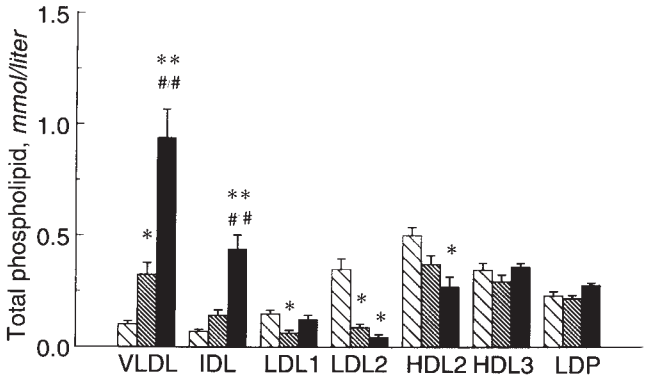

Fig. 6. *Total phospholipid levels (mmol/liter) in lipoproteins and lipid deficient plasma of rats fed standard rat chow (▨, N = 6), rats fed rat chow supplemented with 2% dietary cholesterol (▩, N = 4), and rats fed rat chow supplemented with 2% dietary cholesterol plus 0.5% cholic acid (■, N = 4).* Data are mean ± SEM. Symbols are in Fig. 5.

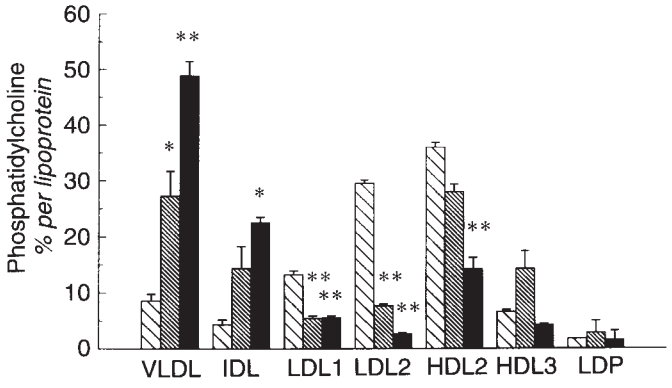

Fig. 7. *Phosphatidylcholine (PC) levels (% of total PC) in lipoproteins and lipid deficient plasma of rats fed standard rat chow (▨, N = 3 × 3), rats fed rat chow supplemented with 2% dietary cholesterol (▩, N = 2 × 2), and rats fed rat chow supplemented with 2% dietary cholesterol plus 0.5% cholic acid (■, N = 2 × 2).* Data are mean ± SEM. Symbols are in Fig. 5.

marked hyperlipidemia ($P < 0.01$ vs. both other groups; Table 3). Cholesterol and phospholipid distribution were affected by dietary cholesterol supplementation alone. VLDL cholesterol and phospholipid concentrations were increased ($P < 0.05$ vs. control) whereas in the LDL2 particles cholesterol and phospholipid levels were reduced ($P < 0.05$; Figs. 5 and 6). Dietary supplementation with cholesterol as well as cholic acid, accentuated the changes observed with cholesterol supplementation only. VLDL and IDL cholesterol and phospholipid levels were markedly increased (all $P < 0.01$), whereas LDL2 and HDL2 cholesterol and phospholipid levels were decreased ($P < 0.05$; Figs. 5 and 6). The relative distribution of total phospholipid in the four phospholipid species was not significantly affected by dietary cholesterol supplementation with or without cholic acid, with the exception of a small decrease in sphingomyelin (Table 3).

PC was significantly increased in the VLDL fraction ($P < 0.05$), and decreased in both LDL fractions ($P < 0.01$) in the rats fed with dietary cholesterol only. PC was markedly increased in the VLDL ($P < 0.01$) and IDL fractions ($P < 0.05$), and decreased in both LDL and the HDL2 fraction (all $P < 0.01$) in the rats fed cholesterol with cholic acid (Fig. 7). There was some increase in LPC content of VLDL and IDL in the groups with dietary cholesterol, particularly in the groups with cholic acid supplementation ($P < 0.05$). However, more than 70% of LPC was localized in HDL3 and the LDP fraction in the control and cholesterol-fed groups, and more than 50% in the cholesterol + cholic acid group (Fig. 8).

Thus in all groups studied the distribution of PC, quantitatively the major phospholipid, correlated with that of plasma cholesterol (cf. Figs. 1 and 3 as well as 5 and 7), whereas LPC was primarily bound to HDL3 and the LDP fraction in normoalbuminemic animals, with or without hyperlipidemia, and primarily bound to lipoproteins, in particular LDL1, LDL2 and HDL2, in hypoalbuminemic animals (cf. Figs. 4 and 8).

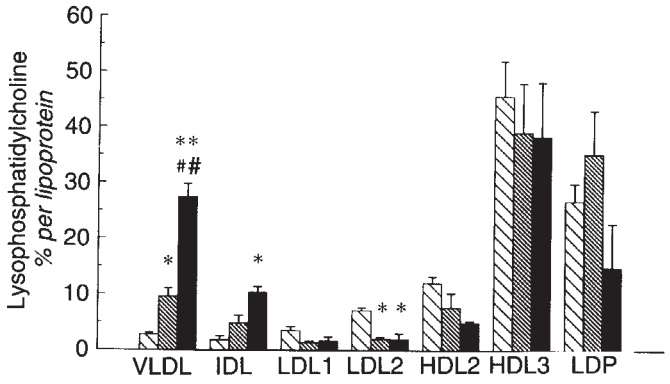

Fig. 8. *Lysophosphatidylcholine (LPC) levels (% of total LPC) in lipoproteins and lipid deficient plasma of rats fed standard rat chow (▨, N = 3 × 3), rats fed rat chow supplemented with 2% dietary cholesterol (▧, N = 2 × 2), and rats fed rat chow supplemented with 2% dietary cholesterol plus 0.5% cholic acid (■, N = 2 × 2).* Data are mean ± SEM. Symbols are in Fig. 5.

Discussion

Abnormal lipoprotein composition may contribute to the high incidence of atherosclerosis in patients with the nephrotic syndrome [21] and to the further deterioration of their renal function [22]. The low HDL2 levels observed in some nephrotic patients [5, 6, 23] may be linked to several defects in the so-called reverse cholesterol transport. One of these putative defects is a low cholesterol esterification rate [7, 8]. Lecithin: cholesterol acyl transferase (LCAT) is a key enzyme for the esterification of cholesterol in plasma [1].

In the present study, we demonstrate that LCAT activity measured by esterification of endogenous lipoprotein cholesterol was not decreased in nephrotic rats, and that in fact LCAT activity measured with excess exogenous substrate was increased. The latter has also been observed in patients with the nephrotic syndrome [9]. Reduced fractional esterification rates (that is, the % of plasma free cholesterol esterified per hour) have been reported in nephrotic patients [7] and rats [10], but this is because total unesterified ("free") cholesterol levels are increased due to the increase in total lipoprotein mass. Our results in adriamycin-induced nephrosis indicate a normal molar (as opposed to fractional) esterification rate.

It is not clear why the increased LCAT activity in nephrotic plasma did not result in an increase in molar esterification rate. It has been suggested that low LCAT activity in the nephrotic syndrome may be linked to depletion of albumin [7], the acceptor of the reaction product, LPC [13]. However, our finding of an increased esterification rate in the NAR argues against this possibility. Apparently albumin is not the obligatory acceptor of LPC (*vide infra*). It is possible that HDL derived from NS rats is not as good a substrate as HDL from NAR, and that this difference is somehow related to the presence of proteinuria in the NS rats. Whether this also holds for nephrotic patients is unknown. An explanation for the reduced HDL2 levels observed in some nephrotic patients may be the reported increased plasma cholesterol ester transfer protein (CETP) activity [9, 24, 25]. CETP activity is virtually absent in rats [26].

LPC has been reported to constitute about 20 to 30% of total phospholipid in rat plasma [10, 13, 27]. In the present study a similar value was found in most groups. Note that these values are considerably higher than the value of about 9% observed in human plasma [13, 28, 29]. In plasma from control animals more than 80% of LPC was bound to the albumin-containing fractions (LDP and HDL3) as has been shown previously in normal human plasma [29]. More than 50% of LPC was found in LDP and HDL3 from normoalbuminemic rats with dietary-induced hyperlipidemia. In contrast, in both NAR and hypoalbuminemic NS rats, 80% of the LPC was bound to lipoproteins rather than to lipoprotein-deficient plasma (LDP) and HDL3. Thus albumin is apparently not the obligatory acceptor of LPC, and the alteration in LPC distribution in the hypoalbuminemic state is not simply the consequence of hyperlipidemia. Indeed, the alteration in LPC distribution was similar in NAR and NS rats despite the fact that the degree of hyperlipidemia was much more severe in the NS rats. Plasma albumin levels were somewhat reduced in the NS rats; however, the decrease was not as dramatic as in the NAR. Thus there does not seem to be a direct inverse correlation between low albumin levels and the alteration in LPC distribution.

It is not clear how much of the increased LPC level in lipoproteins in hypoalbuminemic plasma is due to an increase in cholesterol esterification due to LCAT activity. Enzymatic modification of lipoproteins by enzymes other than LCAT should be considered. For instance, increased phospholipase A_2 activity may play a role in the conversion of PC to LPC [30]. Both high phospholipase A_2 and high LPC levels are characteristic of oxidative modification of LDL [30]. However, it should be noted that LPC levels were increased in all lipoproteins, including HDL which does not contain apo B, whereas phospholipase A_2 activity appears to be a property of apo B itself [30]. Decreased albumin levels may result in a non-specific shift of LPC to those lipoproteins that are most abundant.

High LPC levels in LDL in hypoalbuminemic states such as the nephrotic syndrome where LPC appears to be predominantly bound to lipoproteins may not be innocuous. LDL oxidized *in vitro* (by incubation with cultured endothelial cells) contains much more LPC than native LDL (16 times as much) [31]. LPC in oxidized LDL is a chemotactic factor for monocytes and probably mediates their entry into the developing atherosclerotic lesion [32]. Decreased arterial relaxation is a characteristic of atherosclerotic arteries [33]. Transfer of LPC from oxidized LDL to endothelial membranes can cause marked impairment of endothelium-dependent arterial relaxation [34, 35]. Pertinent to our findings is the observation that adding albumin to oxidized LDL largely restored endothelium-dependent arterial relaxation [34]. Thus, although high LPC levels in LDL and other lipoproteins do not necessarily imply that these particles have been oxidatively modified, the altered phospholipid composition on the surface of these particles may have a biological effect. It is conceivable that lipoprotein-bound LPC plays a role in the high incidence of ischemic heart disease observed in nephrotic patients [36–38].

In summary, this study presents data indicating that plasma cholesterol esterification is not impaired in rats with nephrotic syndrome as compared to control rats, while LCAT activity measured with excess exogenous substrate is increased. In the presence of hypoalbuminemia, lipoproteins, including LDL, carry more than 80% of LPC as compared to less than 20% in

controls. In the presence of normoalbuminemia, hypercholesterolemia does not induce this alteration in LPC distribution. The altered phospholipid composition of lipoproteins in the hypoalbuminemic state, that is also characteristic of lipoproteins that have been oxidatively modified *in vitro*, has not been reported previously *in vivo*. If similar abnormalities occur in humans with hypoalbuminemia, they may well contribute to the high incidence of atherosclerosis observed in patients with the nephrotic syndrome.

Acknowledgments

Portions of this work were presented at the American Society of Nephrology in Baltimore (MD) on November 16, 1992 and appear in abstract form (*J Am Soc Nephrol* 3:741, 1992). J.A. Joles is supported by the Dutch Kidney Foundation, grant nr. C92.1244 and T.J. Rabelink is supported by a fellowship of the Royal Dutch Academy of Sciences. The plasma apolipoprotein assays and plasma protein electrophoresis were performed by Ms. M.M. Geelhoed-Mieras and Mr. J. Vossen, respectively. We acknowledge their contributions to this study.

Reprint requests to Jaap A. Joles, D.V.M., Ph.D., Department of Nephrology and Hypertension, University Hospital (F03.226), P.O. Box 85500, 3508 GA Utrecht, The Netherlands.

References

1. GLOMSET JA: The plasma lecithin:cholesterol acyltransferase reaction. *J Lipid Res* 9:155–167, 1968
2. EISENBERG S: High density lipoprotein metabolism. *J Lipid Res* 25:1017–1058, 1984
3. FIELDING CJ: The origin and properties of free cholesterol potential gradients in plasma, and their relation to atherogenesis. *J Lipid Res* 25:1624–1628, 1984
4. JOHNSON WJ, MAHLBERG FH, ROTHBLATT GH, PHILLIPS MC: Cholesterol transport between cells and high-density lipoproteins. *Biochim Biophys Acta* 1085:273–298, 1991
5. JUNGST D, CASELMAN WH, KUTSCHERA P, WEISWEILER P: Relation of hyperlipidemia in serum and loss of high density lipoproteins in urine in the nephrotic syndrome. *Clin Chim Acta* 168:159–167, 1987
6. MULS E, ROSSENEU M, DANEELS R, SCHURGERS M, BOELAERT J: Lipoprotein composition and distribution in the nephrotic syndrome. *Atherosclerosis* 54:225–237, 1985
7. COHEN SL, CRAMP DG, LEWIS AD, TICKNER TR: The mechanism of hyperlipidaemia in nephrotic syndrome—Role of low albumin and the LCAT reaction. *Clin Chim Acta* 104:393–400, 1980
8. NAYAK SS, BHASKARANAND N, KAMATH KS, BALIGA M, VENKATESH A, AROOR AR: Serum apolipoproteins A and B, lecithin: cholesterol acyl transferase activities and urinary cholesterol levels in nephrotic syndrome patients before and during steroid treatment. *Nephron* 54:234–239, 1990
9. DULLAART RPF, GANSEVOORT RT, DIKKESCHEI BD, DE ZEEUW D, DE JONG PE, VAN TOL A: Role of elevated lecithin:cholesterol acyltransferase and cholesterol ester transfer protein activities in abnormal lipoproteins from proteinuric patients. *Kidney Int* 44:91–97, 1993
10. SESTAK TL, ALAVI N, SUBBAIAH PV: Plasma lipid and acyltransferase activities in experimental nephrotic syndrome. *Kidney Int* 367:240–248, 1989
11. GHERARDI E, CALANDRA S: Plasma and urinary lipids and lipoprotein during the development of nephrotic syndrome induced in rats by puromycin aminonucleoside. *Biochim Biophys Acta* 710:188–196, 1981
12. JOVEN J, MASANA L, VILLABONA C, VILELLA E, BARGALLÓ T, TRIAS M, FIGUERAS M, TURNER PR: Low density lipoprotein metabolism in rats with puromycin aminonucleoside-induced nephrotic syndrome. *Metabolism* 38:491–495, 1989
13. SWITZER S, EDER HA: Transport of lysolecithin by albumin in human and rat plasma. *J Lipid Res* 6:506–511, 1965
14. VAN TOL A, JANSEN EHJM, KOOMANS HA, JOLES JA: Hyperlipoproteinemia in Nagase analbuminemic rats: Effects of pravastatin on plasma (apo)lipoproteins and lecithin:cholesterol acyltransferase activity. *J Lipid Res* 32:1719–1728, 1991
15. BALDO-ENZI G, BAIOCCHI MR, VIGNA G, ANDRIAN C, MOSCONI C, FELLIN R: Analbuminemia: A natural model of metabolic compensatory systems. *J Inher Metab Dis* 10:317–329, 1987
16. JOLES JA, VAN TOL A, JANSEN EHJM, KOOMANS HA, RABELINK TJ, GROND J, VAN GOOR H: Plasma lipoproteins and renal apolipoproteins in rats with chronic adriamycin nephrosis. *Nephrol Dial Transplant* 8:831–838, 1993
17. VAN TOL A, SCHEEK LM, GROENER JEM: Net mass transfer of cholesteryl esters from low density lipoproteins to high density lipoproteins in plasma from normolipidemic subjects. *Arterioscler Thromb* 11:55–63, 1991
18. GLOMSET JA, WRIGHT JL: Some properties of a cholesterol esterifying enzyme in human plasma. *Biochim Biophys Acta* 89:266–276, 1964
19. STEYRER E, KOSTNER GM: Activation of lecithin-cholesterol acyltransferase by apolipoprotein D: comparison of proteoliposomes containing apolipoproteins D, A-I or C-I. *Biochim Biophys Acta* 958:484–491, 1988
20. MARINETTI GV: Chromatographic separation, identification, and analysis of phosphatides. *J Lipid Res* 3:1–20, 1962
21. BERNARD DB: Extrarenal complications of the nephrotic syndrome. *Kidney Int* 33:1184–1202, 1988
22. MOORHEAD JF, CHAN MK, EL-NAHAS M, VARGHESE Z: Hypothesis: Lipid nephrotoxicity in chronic progressive glomerular and tubulo-interstitial disease. *Lancet* ii:1309–1311, 1982
23. GHERARDI E, ROTA E, CALANDRA S, GENOVA R, TAMBORINO A: Relationship among the concentrations of serum lipoproteins and changes in their chemical composition in patients with untreated nephrotic syndrome. *Eur J Clin Invest* 7:563–570, 1977
24. MOULIN P, APPEL GB, GINSBERG HN, TALL AR: Increased concentration of plasma cholesteryl ester transfer protein in nephrotic syndrome: Role in dyslipidemia. *J Lipid Res* 33:1817–1822, 1992
25. SUBBAIAH PV, RODBY RA: Abnormalities in the synthesis and transfer of cholesteryl esters in the nephrotic syndrome. (abstract) *J Am Soc Nephrol* 1:343, 1990
26. HA YC, BARTER PJ: Differences in plasma cholesterol ester transfer activity in sixteen vertebrate species. *Comp Biochem Physiol* [B] 71:265–269, 1982
27. NEWMAN HAI, LIU C-T, ZILVERSMIT DB: Evidence for the physiological occurrence of lysolecithin in rat plasma. *J Lipid Res* 2:403–411, 1961
28. BAGDADE JD, SUBBAIAH PV: Abnormal high-density lipoprotein composition in women with insulin-dependent diabetes. *J Lab Clin Med* 113:235–240, 1989
29. GLOMSET JA: Further studies on the mechanism of the plasma cholesterol esterification reaction. *Biochim Biophys Acta* 70:389–395, 1963
30. PARTHASARATHY S, BARNETT J: Phospholipase A_2 activity of low density lipoprotein: Evidence for an intrinsic phospholipase A_2 activity of apoprotein B-100. *Proc Natl Acad Sci USA* 87:9741–9745, 1990
31. JOUGASAKI M, KUGIYAMA K, SAITO Y, NAKAO K, IMURA H, YASUE H: Suppression of endothelin-1 secretion by lysophosphatidylcholine in oxidized low density lipoprotein in cultured vascular endothelial cells. *Circ Res* 71:614–619, 1992
32. QUINN MT, PARTHASARATHY S, STEINBERG D: Lysophosphatidylcholine: A chemotactic factor for human monocytes and its potential role in atherogenesis. *Proc Natl Acad Sci USA* 85:2805–2809, 1988
33. BOSSALLER C, HABIB GB, YAMAMOTO H, WILLIAMS C, WELLS S, HENRY PD: Impaired muscarinic endothelium-dependent relaxation and cyclic guanosine 5'-monophosphate formation in atherosclerotic human coronary artery and rabbit aorta. *J Clin Invest* 79:170–174, 1987
34. KUGIYAMA K, KERNS SA, MORRISETT JD, ROBERTS R, HENRY PD:

Impairment of endothelium-dependent arterial relaxation by lysolecithin in modified-low density lipoproteins. *Nature* 344:160–162, 1990

35. MANGIN JR, EL, KUGIYAMA K, NGUY JH, KERNS SA, HENRY PD: Effects of lysolipids and oxidatively modified low density lipoprotein on endothelium-dependent relaxation of rabbit aorta. *Circ Res* 72:161–166, 1993
36. BERLYNE GM, MALLICK NP: Ischemic heart disease as a complication of the nephrotic syndrome. *Lancet* ii:399–400, 1969
37. CURRY RC JR, ROBERTS WC: Status of the coronary arteries in the nephrotic syndrome. *Am J Med* 63:183–192, 1977
38. ORDONEZ JD, HIATT R, KILLEBREW E, FIREMAN B: The risk of coronary artery disease among patients with the nephrotic syndrome. *Kidney Int* 44:638–642, 1993